OPTIMUM FOR EXTRACT PROCESSING OF STILBENE GLUCOSIDE FROM POLYGONUM
                              MULTIFLORUM
[He Shou Dao Zhong Er Ben Yi Xi Gan Ti Qu Gong Yi You Xuan Ji Pao Zhi
                 Dui Qi Han Liang De Ying Xiang]

                        AI-DI QI, et al.

            UNITED STATES PATENT AND TRADEMARK OFFICE
            Washington, D.C.   November 2009

Translated by: FLS, Inc.

Author: QI, AI-DI

Author's Affiliation: DEPARTMENT OF CHINESE MATERIAL MEDICA, TIANJIN
                         COLLEGE OF TCM, TIANJIN 300193, CHINA

Ai-Di Qi
(DEPARTMENT OF CHINESE MATERIAL MEDICA, TIANJIN COLLEGE OF TCM,
TIANJIN 300193)

Summary: AIM. Optimum extract processing of stilbene glucoside from
polygonum multiflorum. Stilbene glucoside content is compared before
and after preparation. METHOD. Orthogonal testing is used. HPLC is
used to determine the stilbene glucoside content in polygonum
multiflorum. RESULTS. The optimum extraction technique of stilbene
glucoside form polygonum multiflorum is: using 6.0 times the
medicinal material is a significant affecting factor. The difference
in stilbene glucoside content in the polygonum multiflorum before and
after optimization was drastic.
Key words: Polygonum multiflorum Thunb., stilbene glucoside,
orthogonal test, processing

Polygonum multiflorum is a dried root of the polygonaceae plant,
Polygonum multiflorum Thunb. There are different fresh and prepared

---

* Numbers in the margin indicate pagination in the foreign text.

parts of the polygonum multiflorum, depending on the processing method used. Fresh polygonum multiflorum is bitter to the taste, is astringent, is characteristically warm, and is effective in moisturizing the intestines, removing toxins, and preventing malaria. Perpared polygonum multiflorum is bitter to the taste, is sweet, is astringent, is characteristically warm, and is effective in compensating the liver and kidneys, providing essence to the blood, strengthening the bones, and blackening the hair[1]. Modern pharmacology has proven that the stilbene glucoside compounds in polygonum multiflorum and prevent aging, reduce cholesterol, improve immunity function, prevent arteriosclerosis, and protect the liver[2]. This research utilizes orthogonal testing to determine the optimal technique for extracting stilbene glucoside from polygonum multiflorum. And, based on this, compares the stilbene glucoside content before and after the preparation thereof.

1. Instruments and Reagents

HP110 high performance liquid chromatography system, 1314-UV modifiable wave length detector, HP Rev. A. 0501 chemical work station. The acetonitrile is chromatographically pure, the water ultra—pure water, and the methyl alcohol and ethyl alcohol are analytically pure. A stilbene glucoside control product is provided (Chinese Medicine and Biological Products Verification Office, lot number: 0844 – 9802). The fresh polygonum multiflorum medicine and

prepared polygonum multiflorum were purchased from different locations.

2. Spectrometry Conditions

Supelcosil™ LC$_{18}$ spectrometry columns (5 μm, 4.6 mm x 250 mm). The liquid flow phase acetonitrile – water (18 : 82). Flow rate: 1.0 mL/min. Measurement wave length: 320 nm. Column temperature: 35°C. stilbene glucoside control and the fresh polygonum multiflorum and prepared polygonum multiflorum spectrum are shown in Figure 1.
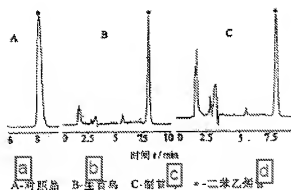

Figure 1. HPLC Diagram

Key:
a) A — Control product
b) B – Fresh polygonum multiflorum
c) C – Prepared polygonum multiflorum
d) * – Stilbene glucoside

3. Orthogonal Test Design

Based on the physical and chemical properties of the active ingredients in polygonum multiflorum, as well as the information in the referenced documents[3] and experimental results, the affecting factors thereof are primarily the concentration of the alcohols, the amount of alcohols added, and the extraction time, etc. Therefore, a $L_9(3^4)$ design was prepared. The factor levels thereof are shown in

4

Table 1 and the results are shown in Table 2. A analysis of variance was obtained using statistical processing[4]. See Table 3.

Table 1. Table of Test Factor Levels

Level	A. Alcohol concentration (%)	B. Ethyl alcohol dosage	C. Heat time (min)
1	50	6.0	30
2	75	8.0	60
3	95	10.0	120

Key:
a) Level
b) A. Alcohol concentration (%)
c) Factors
d) B. Ethyl alcohol dosage
e) C. Heat time (min)

Table 2. Orthogonal Test Solutions and Results

Test number	A	B	C	Stilbene glucoside content in polygonum multiflorum (mg/g)
1	1	1	1	12.12
2	1	2	2	11.97
3	1	3	3	11.61
4	2	1	2	11.38
5	2	2	3	11.16
6	2	3	1	11.43
7	3	1	3	10.49
8	3	2	1	10.84
9	3	3	2	10.23
K_1	35.70	33.99	34.39	
K_2	33.97	33.97	33.58	
K_3	31.56	33.27	33.26	
R	4.14	0.72	1.13	

Key:
a) Test number
b) Stilbene glucoside content in polygonum multiflorum (mg/g)

5

<div align="center">Table 3. Analysis of Variance</div>

a) 变异源	b) 离差平方和	c) 由度	d) 均方	F 值 e)	P
A	2.88	2	1.44	96	<0.05
B	0.11	2	0.055	4	
C	0.23	2	0.115	8	
误差 f)	0.03	2	0.015		

$F_{0.05}(2,2)=19.0$ $F_{0.01}(2,2)=99.0$

Key:
a) Variance source
b) Sum of squares
c) Degree of freedom
d) Mean square
e) F value
f) Error

It can be seen that the level of affect of the factors on the effectiveness of the extract is in sequence A > C > B. Factor A clearly exhibits significant difference, of which the greatest affecting factor is the concentration of the ethyl alcohol. Next is extraction time. The amount of ethyl alcohol used affects this in a smaller scale. Therefore, the optimal level combination of the factors is $A_1B_1C_1$.

Experimental Proof: Precisely weigh 2 g of prepared polygonum multiflorum powder (passed through a 60 mesh sieve). Process according to the optimal level combination. The results show that the stilbene glucoside content practically matches the data of No. 1 in Table 2. Therefore, the proven process for polygonum multiflorum extraction is: Grind the polygonum multiflorum into a powder (pass through a 60 mesh sieve). Heat reflux with 6.0 times the amount 50% ethyl alcohol for 30 min.

4. Content Determination

4.1. Graphing standard curves:

Precisely weigh 3.6 mg of stilbene glucoside control product. Place in a 10 mL flask. Add methyl alcohol to dissolve and dissolve to graduation to obtain the control product solution. Accurately weigh 0.1, 0.2, 0.5, 1.0, 1.5, and 2.0 mL, respectively, and place in 5 mL flasks. Add methyl alcohol at constant volume until graduation. Evenly mix. Filter with a 0.45 μm micro pore filter membrane. Prepare a 10 μL sample. Record the spectrometry. Statistically process the peak areas of each corresponding concentration to obtain a regression equation: $Y = 27,524.6X - 116.6$, $r = 0.9998$. The linear relationship of the samples around 0.072 - 1.440 μg is good.

4.2. Preparation of the Sample Test Solution:

Precisely weigh 2 g each of polygonum multiflorum powder (passed through a 60 mesh sieve) from different sources. Place in a round bottom flask. Add in 6.0 times the amount of 50% ethyl alcohol. Heat reflux the aqueous solution for 30 minutes. Filter. Vacuum evaporate the filtered solution. Dissolve the residue with methyl alcohol and transfer into a 10 mL flask. Keep at constant volume until graduation. Mix evenly and let settle. Filter using a 0.45 μm micro pore filter membrane and set aside for future use.

4.3. Accuracy Test:

Precisely draw 10 μL of the control product solution. Perform 6 continuous samples. The peak area RSD is 1.46%.

4.4. Repeatability Test:

Precisely weigh six parts from the same batch. Perform the same steps for the preparation of the sample test solution. Prepare a 10 µL sample. Record the spectrometry. The peak area RSD is 1.38%.

4.5. Stability Test:

Prepare the control product solution. Sample 10 µL every 30 minutes on the different concentrations. Continue for 4 hours. The results show the peak area integral within 4 hours was basically stable. The peak area RSD is 1.42%.

4.6. Sample Recovery Rate Test:

Precisely weigh six parts from the same batch. Add a suitable amount of control product to each. Perform the same steps for the preparation of the sample product. Perform a spectrometric analysis and calculation. The average recovery rate is 100.05%, RSD = 1.01%.

4.7. Sample Product Content Determination.

Prepare 10 µL of each sample test solution. Determine the stilbene glucoside content. See Table 4 for the results.

Table 4. Stilbene glucoside content determine in polygonum multiflorum from different sources (n = 4)

a) 品種	b) 品名	c) 含乙醇苷(mg/g)	RSD(%)
黑龙江省药材公司	生品	22.66	2.12
	制品	8.28	1.56
河北省药材公司	生品	13.14	2.34
	制品	4.04	2.46
山东省药材公司	生品	33.77	2.23
	制品	10.49	2.08
天津中医学院门诊部	生品	48.15	1.41
	制品	12.21	1.73

Key:
a) Source
b) Product name
c) Stilbene glucoside (mg/g)
d) Heilongjiang Province Medicinal Materials Company
e) Hebei Province Medicinal Materials Company
f) Shandong Province Medicinal Materials Company
g) Tianjin College of Chinese Medicine, Out-patient Department
h) Fresh product
i) Prepared product

5. Conclusion

5.1. Comparing the polygonum multiflorum before and after preparation, the difference in stilbene glucoside content was quite significant. This is possibly related to the attenuated efficiency during the preparation of Chinese medicine.

5.2. Stilbene glucoside content in the polygonum multiflorum from different sources varied as much as 2 − 3 times. This shows that there are different levels of quality of the medicinal materials on the market. Therefore, the preparation method for polygonum multiflorum must be further standardized. In other words, a unified quality control standard must be established to ensure the use of the drug is reasonable and safe.

Reference Documents:

[1] Su, Wei; Guo, Qun. Research on Modern Pharmacology of Polygonum
    Multiflorum [J], Chinese Herbal Medicine, 1997, 28 (2): 119
[2] Li, Yusu; He, Xunhua. Modern Research and Development of
    Polygonum Multiflorum [J], Chinese Traditional Patent Medicine,
    1997, 19 (5): 37
[3] Yuan, Hailong; He, Chengshan; Xiao, Xiaohe. Highly Effective
    Capillary Electrophoresis Methods for Determining Stilbene
    Glucoside Content in Polygonum Multiflorum [J]. Pharmaceutical
    Journal of the Chinese People's Liberation Army, 2000, 16 (3):
    151 - 154
[4] He, Shilin. Traditional Chinese Medicine Research Design and
    Statistical Methods [M], Changsha: Hunan Science and Technology
    Publishers, 1989